

## INTEGRATION OF CELLULAR, ORGANISMAL, AND ECOLOGICAL ASPECTS OF SALT AND WATER BALANCE

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Crustaceans occupy habitats ranging from hypersaline through freshwater and terrestrial, and differing in variability of temperature, salinity, and moisture over periods of hours to months. Even in stable, isosmotic environments, they must expend energy in maintaining their internal osmotic and ionic milieu. The impetus for maintaining a constant internal environment can be traced to the deleterious effects of volume changes in individual cells, with the resulting structural and functional changes in proteins. Organisms employ a combination of inorganic ion transport and organic osmolyte deployment to match intracellular and extracellular osmolalities, thus reducing the energy required to maintain cell volume. In multicellular animals, such as crustaceans, changes in external salinity are countered by two strategies. In the first, the osmolality of the internal fluid tracks that of the external medium, transferring osmotic work to the individual cells. The second strategy involves regulation of the osmolality of the internal fluid. The work of osmo- and iono-regulation is concentrated in surfaces in contact with the external medium, such as the integument and gut. Water regulation is relegated to specialised excretory organs, such as the antennal gland, and ion transport to spatially restricted and specialised tissues, such as the gills of crabs and salt glands of *Artemia*. A number of ion transport systems important in iono- and osmoregulation have been identified in crustaceans. Many of these systems are integral to acid-base balance and nitrogen excretion as well. In addition to sharing common enzymatic pathways, ion regulation frequently occurs on morphological structures with multiple functions. Thus, the gills of decapod crabs serve gas exchange, nitrogen excretion, and ion exchange. Constraints caused by the limited number of physiological mechanisms and morphological options result in integrated responses of salt and water balance, acid-base balance, gas exchange and intermediary metabolism. Examples are given from aquatic decapods. Some terrestrial crabs employ a single behavioural modification, urine recycling at the gill, that addresses several concurrent physiological problems, such as ion depletion, water limitation and nitrogen excretion. In crustaceans in general, behavioural adaptations permit occupation of habitats that would be untenable based on physiological and morphological capabilities alone. To describe the water and salt balance of a particular organism, it is therefore necessary to integrate information from cellular mechanisms, organismal responses, and field observations. □ *Osmoregulation, ionoregulation, ion transport, volume regulation, crustaceans, terrestrial crabs, osmolytes.*

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Cell function is supported only within a narrow range of ionic and osmotic conditions. If uncompensated, chemical changes resulting from metabolic processes would inexorably move the cell from the dynamic equilibria optimal for enzyme function. Such constancy of the internal environment that exists must be maintained through dynamic, energy-requiring transport of ions, aided, and also thwarted, by passive water and ion fluxes. The energetic cost of osmo- and iono-regulation differs in degree, but is incurred by marine, brackish, freshwater, and terrestrial organisms alike.

The physical laws of thermodynamics ulti-

mately dictate the movements of solvent and solutes across the cell membrane between the external and internal media. The forces involved, and the equations that describe them, are well reviewed in Mantel and Farmer (1983), with additional information on water fluxes in a terrestrial environment found in Greenaway (1988).

Enhanced fitness of organisms that maintain proper solute and water relations in the interior of individual cells drives evolution of osmo- and iono-regulatory systems. When cells are exposed to fluids that are anisomotic (not isosmotic) to the cell's interior; they tend to shrink

or swell, depending on whether the fluids are hyper- or hypo-osmotic to the cytoplasm. Cell volume can be restored by the active extrusion or uptake of inorganic solutes, such as  $\text{Na}^+$ , and by manipulating the concentration of organic osmolytes, such as free amino acids (Hoffman and Simonsen, 1989; Chamberlin and Strange, 1989; Pierce, 1982). Cells of organisms that show volume regulation are able to return to their normal volume by matching the internal osmotic pressure to the new extracellular regime.

Volume regulation under anisomotic conditions involves a number of processes. In the short term, inorganic ions are pumped into or out of the cell to bring the fluid compartments on either side of the cell membrane into osmotic balance, restoring cell volume. However, the conformation of proteins, and hence their function, is intimately dependent on the presence and concentration of particular ions. Therefore, for long-term osmotic adjustments, organisms employ a variety of organic osmolytes. Organic osmolytes, although metabolically expensive to deploy, have a distinct advantage over inorganic ions in that they do not compromise protein function even when present in high concentrations (Yancy *et al.*, 1982).

Organic osmolytes are selected for their compatibility with enzyme and other physiological functions, and on the basis of their metabolic or environmental availability (Hochachka and Somero, 1984; Gilles and Pequeux, 1983; Goolish and Burton, 1989). Across the entire spectrum of living creatures, organic osmolytes include sugars, polyols, amino acids, methylamines, and urea. Crustaceans employ all but the last two, with non-essential free amino acids being most common (Gilles and Pequeux, 1983; Chamberlin and Strange, 1989).

For both rapid volume adjustments and general cell function, movement of inorganic ions occurs through ion-specific channels and pumps, and via a variety of exchangers and cotransporters. Paramount among the transport systems is  $\text{Na}^+/\text{K}^+$ -ATPase, responsible for pumping  $\text{Na}^+$  from the cell. The inwardly-directed electrochemical gradient for  $\text{Na}^+$  thus created provides the motive force for the movement of other ions against their concentration gradients. The coupled movement of  $\text{Cl}^-$  and  $\text{Na}^+$  is an example of cotransport. Most cotransport systems described so far involve coupled transport of cations and anions whose charge balance is zero. Such systems are electroneutral, or electrically 'silent'. Their operation neither affects the membrane

potential nor is affected by it (Hoffman, 1986). Like cotransport systems, exchange systems (e.g.  $\text{HCO}_3^-$  for  $\text{Cl}^-$ ) can also be electroneutral.

Because ion transport and water balance are problems around which life evolved, the mechanisms are fairly universal. Ion transport mechanisms identified in other organisms are found to operate in crustacean tissue as well (Table 1)(Chamberlin and Strange, 1989). Some of the conflicting data surrounding the  $\text{Na}^+/\text{H}^+$  exchanger in crustaceans may be resolved, now that the presence of an electrogenic,  $2\text{Na}^+:\text{H}^+$  exchanger has been identified, both in crab gill (*Callinectes sapidus*, Towle and Baksinski, 1989) and in lobster antennal gland (*Homurus americanus*, Franco and Ahearn, 1989).

Some progress is being made in the search for the signals and sensors whereby individual cells detect volume changes (Chamberlin and Strange, 1989). Ion channels have been identified that respond directly to the changes in hydrostatic pressure resulting from the efflux or influx of water during anisomotic conditions by altering the transport of specific ions. Such stretch-activated ion channels respond to changes as small as 5 mmHg. The amount of membrane stretch resulting from a volume increase of even 1% would be sufficient to activate the  $\text{K}^+$  channel in *Necturus* proximal tubule cells (Sackin, 1987). The cytoskeleton may play a role in stimulating stretch activated channels by amplifying volume-induced stretch. Stretch inactivated channels have been identified coexisting in the same neurons as stretch activated channels (Morris and Sigurdson (1989). Chamberlin and Strange (1989) speculate that coordinated activation of transporters active in volume regulation and deactivation of resting conductances would permit more rapid and energetically efficient volume regulation.

Once cells receive input signalling a change in osmotic conditions, either physically, through hydrostatically-transmitted information, e.g. stretch activated receptors, or chemically, e.g. by a variety of intracellular second messengers which integrate the cells osmoregulatory response.

Several intracellular signals have been implicated in cell volume regulation, including cyclic AMP, leukotrienes, protein kinases, and  $\text{Ca}^{++}$ , with the possible involvement of calmodulin (Chamberlin and Strange, 1989). Current evidence confirms that cyclic AMP is an intracellular modulator of ion transport in crustaceans as well (Bianchini and Gilles, 1990).

The permeability of cells to water and ions is affected by the kinds and quantity of lipids present in the cell membrane. Changes in the quality and quantity of membrane and blood lipids, including cholesterol, correlate with changes in permeability (Chappelle and Benson, 1986; Spaargaren and Mors, 1985; Subramanyam and Krishnamoorthy, 1983) and link osmoregulation with lipid metabolism.

Since the metabolic processes discussed so far require energy, ionic and osmo-regulation are interdependent with respiration and those physical and biotic factors which effect it.

In the context of multicellularity, the interaction of an organism with its environment is changed in fundamental ways. For a given amount of cytoplasm, the proportion of membrane exposed to the environment is reduced, e.g. every organism small enough to depend solely on diffusion for transport of oxygen and nutrients, and for release of metabolic wastes (approximately 1 mm maximum effective thickness, Graham, 1988) only needs to control permeability and to transport ions over a small proportion of its total plasma membrane.

Concurrent with evolution of larger body form is the development of interior fluid reservoirs and circulatory systems to enhance transport of gases and solutes. The major and initial response to variations in the environment is still confined to the integument, but the organism has the option of controlling the composition of the interior fluid spaces. Again, the driving force is the maintenance of an intracellular ionic and osmotic environment optimal for cell function. Crustaceans employ two options in dealing with salinity fluctuations. One is to allow the fluid bathing the cells, the haemolymph, to track external salinity, thus transferring the work required during anisotonic regulation to the cell. Alternatively, they may manipulate haemolymph composition during salinity changes to minimise the osmotic work that the cells must do. The former option, osmoconformity, restricts organisms to habitats where the osmotic concentration is fairly high and stable, or commits them to volume regulation by cells during adverse osmotic conditions. The second pattern, osmoregulation, permits occupation of ionically or osmotically extreme or variable habitats. Osmoregulation and osmoconformity can exist in the same organism over different parts of the salinity scale. For instance, in marine organisms occasionally exposed to fresh water input, conforming at high salinities and hyper-osmoregulating at low salinities

would be adaptive. However, the impetus for maintaining a constant ionic concentration in coelomic fluids is still to reduce the need for volume regulation by individual cells. The regulatory options adopted by different organisms are reflected in the familiar categories of osmoregulation: osmoconformity, hyperregulation, and hyporegulation, and the combinations thereof (Mantle and Farmer, 1983). As external salinity varies, the effects on cell volume will depend on the type of osmoregulation available, both at the organism's surface and at the cell (Gilles and Pequeux, 1983).

In larger organisms, and those adapted to variable habitats, tissue that regulates transport of ions and water is highly localised, with the rest of the surface fairly impermeable, thus passively restricting exchange with the environment. Rather than being uniformly deployed in the cell membrane, certain ion-transporting enzymes are restricted to or concentrated on a particular surface. For instance,  $\text{Na}^+/\text{K}^+$ -ATP-ase is highly concentrated in the basal-lateral membrane of the gill epithelial cell, where it apparently functions to transport cations, including  $\text{NH}_4^+$ , between the cell interior and the haemolymph (Table 1) (Towle and Kays, 1986; Towle and Holleland, 1987). Both the activity and quantity of this enzyme increase dramatically with changes in salinity (Kirschner, 1979; Towle, 1984). On the apical surface,  $\text{Na}^+$  is exchanged for  $\text{H}^+$  (Table 1).

Commonly, respiratory, feeding, and locomotory functions are combined at the same surface, as is ion and solute transfer. Water flow created by feeding and locomotory currents enhances exchange of solutes across the integument by reducing the boundary layer. Most aquatic phyla are dependent on integumental gas exchange at some point in their life history (Graham, 1988). Among crustaceans, individuals depend on integumental exchange during development as eggs and larvae.

Salt and water balance are intertwined with acid-base balance, gas exchange, and through changes in free amino acid concentrations, intermediary metabolism and fluxes of ammonia. The links are forged by the commonality of biochemical mechanisms and morphological structures involved in these processes. Synthesis and catabolism of free amino acids during fluctuations in salinity links osmoregulation, nitrogen metabolism, and excretion. Use of common pathways also interconnects ion regulation and acid-base balance.

TABLE 1. Examples of ion transport pathways identified in crustaceans.

TRANSPORT MECHANISMS	SPECIES	LOCALISATION	REFERENCES
Na <sup>+</sup> /K <sup>+</sup> -ATPase	<i>Artemia salina</i>  <i>Callinassa jamaicense</i> <i>Callinectes sapidus</i>  <i>Carcinus maenas</i>  <i>Eriocheir sinensis</i> <i>Gecarcinus lateralis</i> <i>Homarus gammarus</i> <i>Procambarus clarkii</i> <i>Uca minax</i> <i>U. pugilator</i>  <i>U. pugnax</i> <i>U. tangeri</i>	metepipodites + head, maxillary gland, gut larval salt glands larval salt glands larval stages gills NH <sub>4</sub> <sup>+</sup> activation NH <sub>4</sub> <sup>+</sup> substitutes for K <sup>+</sup>  basolateral membrane gills basolateral membrane  gills larval stages antennal gland  gills 5 and 6 gills 5 and 6 basolateral gills 5,6	Holliday, 1985a  Ewing <i>et al.</i> , 1974 Lowy and Conte, 1985 Felder <i>et al.</i> , 1986 Mantel and Olson, 1976 Towle <i>et al.</i> , 1976 Towle and Holleland, 1987 Neufled <i>et al.</i> , 1980 Towle and Kays, 1986 Siebers <i>et al.</i> , 1982 Towle and Kays, 1986 Pequeux and Gilles, 1977 Mantel and Olson, 1976 Thuet <i>et al.</i> , 1988 Sarver and Holliday, unpubl. Wanson <i>et al.</i> , 1984 Graszynski and Bigalke, 1987 D'Orazio and Holliday, 1985 Holliday, 1985b Graszynski and Bigalke, 1987
Na <sup>+</sup> /H <sup>+</sup>	<i>C. sapidus</i>  <i>Corophium curvispinum</i> <i>Eriocheir sinensis</i>  <i>Uca tangeri</i>	gills, apical amilioride sensitivity gill membrane vesicles	Pressley <i>et al.</i> , 1981 Burnett and Towle, 1990 Taylor and Harris, 1986 Graszynski and Bigalke, 1987 Pequeux and Gilles, 1988 Graszynski and Bigalke, 1987
2 Na <sup>+</sup> /H <sup>+</sup>	<i>C. sapidus</i> <i>Homarus americanus</i>	posterior gill vesicles antennal gland vesicles	Towle and Baksinski, 1989 Franco and Ahearn, 1989
Na <sup>+</sup> /Na <sup>+</sup>	<i>C. sapidus</i>		Pressley <i>et al.</i> , 1981
Na <sup>+</sup> channel	<i>Uca tangeri</i>		Graszynski and Bigalke, 1987
Carbonic anhydrase (CO <sub>2</sub> +H <sub>2</sub> O ⇌ H <sup>+</sup> +HCO <sub>3</sub> <sup>-</sup> )	<i>C. sapidus</i>  <i>Cardisoma carnifex</i> <i>Cardisoma guanhumi</i> <i>Gecarcinus lateralis</i> <i>Homarus gammarus</i>	gills gills 6-8 cytoplasmic+membrane gills gills 6-8 gills 8 and 9	Neufeld <i>et al.</i> , 1980 Burnett <i>et al.</i> , 1981 Henry and Cameron, 1982 Henry, 1988 Randall and Wood, 1981 Henry and Cameron, 1982 Henry and Cameron, 1982 Thuet <i>et al.</i> , 1988
Cl <sup>-</sup> channel	<i>Carcinus maenas</i> <i>Uca tangeri</i>	basal	Lucu and Siebers, 1987 in Graszynski and Bigalke, 1987
Cl <sup>-</sup> /HCO <sub>3</sub> <sup>-</sup>	<i>Callinectes sapidus</i>	gill	Burnett and Carroll, 1989

## AMMONIA

If the transport mechanisms of the gill are overwhelmed by the speed or amount of salinity change, the haemolymph concentration will change. To minimise the work that must be done to maintain volume in the face of water influx, intracellular osmotic concentration is lowered both by increasing inorganic ion excretion, and by exporting or metabolizing some of the free amino acids (FAA). Metabolism of the FAA releases ammonia, which is transported by the haemolymph, dissolved or incorporated into glutamate (Regnault, 1987), to the gill. There, ammonia is exchanged for  $\text{Na}^+$  (Towle and Holleland, 1987; Lucu *et al.*, 1989), coupling ion uptake and nitrogen excretion. Enhanced rates of ammonia excretion during hypoionic stress have been reported in the shore crab, *Carcinus maenas* (Spaargaren, 1982; Harris and Andrews, 1985), the intertidal prawn, *Palaemon elegans* (Rathke) (Taylor *et al.*, 1987), the shrimp *Crangon crangon* L. (Regnault, 1984), *Panaeus japonicus* Bate (Spaargaren *et al.*, 1982) and several other species (Mantel and Farmer, 1983).

Ammonia excretion can respond rapidly to changes in salinity, e.g. in the intertidal prawn, *Palaemon elegans* (Rathke), which may be subjected to frequent, rapid fluxes in salinity. Declines in FAA in the tail muscle, increases in ammonia excretion and adjustments in  $\text{Na}^+$  concentration all occur within the first two hours following a major salinity drop (Taylor *et al.*, 1987). Similar rapid shifts in ammonia excretion follow transfer to low salinity in the blue crab *Callinectes sapidus* (Rathbun) (Mangum *et al.*, 1976), whose habitat is marked by longer-term seasonal fluctuations in salinity, in addition to storm events. Organisms inhabiting estuaries with large tidal fluxes experience repetitive cycling of salinity. *Crangon crangon* in the Penze estuary, France, has enhanced rates of ammonia excretion during ebb tides, as riverine water lowers the salinity, and lowered rates of excretion during flood tide, as high salinity waters return. The ammonia excretion rate is influenced by the velocity and direction of the salinity change, and by the range of salinities involved, and is greater in the winter. In the laboratory, shrimp exposed to simulated tidal cycles of salinity under winter conditions lose 1.75 times more ammonia than those in constant salinity (Regnault, 1984). In nature, burrowing (Birchard *et al.*, 1982; Zanders and Martelo, 1984) and circatidal activity rhythms (Al-Adhub and Naylor, 1975) may reduce exposure to salinity

changes and minimise the energetic cost of inhabiting a variable environment.

## GAS EXCHANGE

In *Callinectes sapidus*, inorganic ions and  $\text{H}^+$  ions compete for the same site on the haemocyanin molecule, and raise or lower the oxygen affinity, respectively (Mangum, 1986). During hypoionic exposure, enhanced production of ammonia counteracts the lowering of oxygen affinity of haemocyanin caused by reduced  $\text{Ca}^{2+}$  concentration (Weiland and Mangum, 1975). A permanent solution to reduced  $\text{Ca}^{2+}$  concentration in the haemolymph of blue crabs acclimated to low salinity is the synthesis of an alternate haemocyanin subunit, and changes in the proportions of another, such that oxygen affinity is re-established at a new, lower level (Mason *et al.*, 1983; Mangum and Rainer, 1988).

## ACID-BASE BALANCE

The pH of the cell depends on the strong ion difference (SID), as well as on the partial pressure of  $\text{CO}_2$  ( $P_{\text{CO}_2}$ ) and the concentration of weak acids (Stewart, 1978). The major control of pH in water-breathing animals is through adjustments of the SID (the difference between the quantity of fully-dissociated strong base cations and strong acid anions) (Cameron, 1978; Stewart, 1978). As  $\text{Cl}^-$  and  $\text{Na}^+$  are moved across transporting epithelia, possibly in exchange for  $\text{HCO}_3^-$  and  $\text{H}^+$ , pH can change. Protein, amino acid, and ammonia concentrations change as cell volume regulation occurs. Thus, the same systems that are involved in ion regulation affect acid-base balance as well (Cameron, 1978; Hoffman and Simonsen, 1989). Hypersaline exposure can cause acidosis, as metabolic acid production outstrips increases in bicarbonate concentration (Wheatly and McMahon, 1982), and hyposaline conditions may result in alkalosis due to excess base cations (*Carcinus maenas* L., Truchot 1973, 1981; *Callinectes sapidus*, Weiland and Mangum, 1975).

In marine aquatic crabs, the gill plays a major role in ion transport, gas exchange and ammonia excretion, with the role of the antennal gland largely that of water balance, since the urine produced is isosmotic to the blood. Several freshwater crustaceans have evolved the capacity to produce a dilute urine, and the antennal gland in these forms functions remarkably like a vertebrate kidney, playing an enhanced role in ion and acid-base balance and ammonia excretion (Wheatly and McMahon, 1982; Wheatly

and Toop, 1989). Even in these forms, the majority of ion regulation occurs at the gill (Wheatly and Toop, 1989). The gut plays a role in ion regulation and water balance, too, (review, Mantel and Farmer, 1983), but the contribution of the gills is paramount.

How is ion regulation achieved in terrestrial crustaceans, given that the major ion-permeable surfaces, the gills, are no longer immersed in large volumes of water? Are the ion-pumping, nitrogen excreting, acid-base balancing capabilities of the gills lost to the organism? Not necessarily. Precisely because the organisms are in air, voided urine is not lost to the environment, and can be passed to the gill surfaces for ion reclamation (Wolcott and Wolcott, 1985, 1991, and unpubl.). So far, several species have been shown to reclaim ions from the urine during reprocessing at the gill (Table 2). The excretory product ('P') that is eventually released by the animal can be very dilute, comparable in ion concentration to hypoosmotic urine produced by several freshwater crustaceans (Table 2). Reprocessing essentially permits the production of a dilute urine, thus circumventing behaviourally the limited abilities of the antennal gland.

Urine represents the most saline water available to hyperosmoregulating terrestrial crustaceans, and as such, is a vital resource. The red land crab, *Gecarcinus lateralis*, inhabits dry upland burrows, and its water sources are dilute. When ion concentrations in the haemolymph are experimentally depleted, they can be replenished with ions from the diet (Wolcott and Wolcott, 1988). However, even feeding crabs show declines in haemolymph osmolality when the urine is prevented from reaching the gills (Wolcott, 1991). Avoidance of standing water, as in *Gecurcoidea lalandii* (Cameron, 1981) may be an adaptation to prevent accidental dilution of urine in the branchial chamber.

Ion reclamation is not the only function of the gill that is preserved by urine reprocessing. In both *G. lateralis* and the amphibious crab, *Cardisoma guanhumi*, ammonia concentrations increase 14 and 10 fold, respectively, as urine is reprocessed into 'P' (Table 2) (Wolcott, 1991). Not all species that recycle urine use 'P' for nitrogen waste disposal, and nitrogen excretion in species that recycle urine is turning out to be highly variable (Greenaway, pers. comm., and this volume).

In air-exposed crabs, fluid in the branchial chamber, whether water from the environment or urine from the antennal glands, may provide a reservoir for acid-base adjustments in those crabs that can regulate ions (Burnett and McMahon, 1987; Burnett, 1988). *Eurytium albidigitum*, an intertidal osmoconformer, experiences emersion-induced respiratory acidosis, and is inactive when in air. In *Pachygrapsus crassipes*, an ion regulator, such respiratory acidosis is fully compensated, and crabs remain active in air. Unlike *E. albidigitum*, fluid in the branchial chamber of *P. crassipes* rapidly increases in both carbon dioxide and in titratable alkalinity upon air exposure. Burnett (1988) suggests that alkalisation of the branchial chamber fluid maintains a steeper gradient of  $P_{CO_2}$  across the gill, favouring transport of bicarbonate from the haemolymph, and counteracting respiratory acidosis. Haemolymph ion regulation may have evolved in organisms whose variable habitat or vigorous exercise resulted in acid-base imbalance, with concomitant disruption of metabolism and activity (Ballantyne *et al.*, 1987). Ion regulation in turn supported utilisation of brackish, freshwater and terrestrial habitats (Potts and Durning, 1980).

Urine passed to the branchial chamber in the amphibious crab, *Cardisoma guanhumi*, has a high pH, and very high titratable alkalinity, about 20 times greater than sea water (D. Wolcott, unpubl. data, Table 2). This may also assist movement of  $CO_2$  across the gill, a process much slower in air than in water, reducing respiratory acidosis on emersion, and compensating for the hyper-capnic conditions inside the crabs' burrows (Pinder and Smits, 1986).

Adjustment of pH by altering the ratio of cations and anions is more rapid than relying on metabolic removal of lactate after exercise or air exposure. Osmoconformers such as *Eurytium albidigitum* and *Libinia emarginata* apparently do not use ion exchange for pH regulation, and haemolymph acidosis is uncompensated (Burnett and McMahon, 1987; Booth, 1986).

From the complex interplay of ion and osmotic regulation in extracellular fluid and cells, involving ion transport, water fluxes, acid-base balance, oxygen transport, free amino acid pools, and nitrogen excretion, it is plain that the ion- and osmoregulatory abilities of the whole organism cannot be inferred from the abilities exhibited in isolated tissues, or measured under unnatural conditions. Often, abilities of organ-

TABLE 2. Ion reclamation in hypoosmotic urine of Crustacea from fresh water habitats and reprocessed urine of crabs from terrestrial habitats.

		Haemolymph (mmol/L)	Urine (mmol/L)	P (mmol/L)	References
<b>FRESHWATER SPECIES</b>					
<i>Austropotamobius pallipes</i>	Na <sup>+</sup>	208	11	—	Reigel, 1968
<i>Corophium curvispinum</i>	Na <sup>+</sup>	164	15	—	Taylor and Harris, 1986
<i>Gammarus pulex</i>	Na <sup>+</sup>	152	27	—	Lockwood, 1961
<i>Gammarus duebeni</i>	Na <sup>+</sup>	255	83	—	Lockwood, 1961
<i>Goniopsis cruentata</i>	Na <sup>+</sup> Cl <sup>-</sup>	390 370	190 190	— —	Zanders, 1978
<i>Macrobrachium australiense</i>	Cl <sup>-</sup>	150	60	—	Denne, 1968
<i>Procambarus clarkii</i>	Na <sup>+</sup>	185	6	—	Kamemoto <i>et al.</i> , 1966
<i>Pacifastacus leniusculus</i>	Na <sup>+</sup> Cl <sup>-</sup>	195 180	14 3	— —	Pritchard and Kerley, 1970
<b>TERRESTRIAL SPECIES</b>					
<i>Birgus latro</i>	Na <sup>+</sup> Cl <sup>-</sup>	— —	— —	59 74	Greenaway and Morris, 1989
<i>Cardisoma guanhumii</i>	Na <sup>+</sup> Cl <sup>-</sup> NH <sub>4</sub> <sup>+</sup> CO <sub>2</sub> TA pH	336 318 — — — —	— — 0.5 34 48 8.17	86 33 13 6 8 7.67	Wolcott and Wolcott, unpubl.
<i>Gecarcinus lateralis</i>	Na <sup>+</sup> Cl <sup>-</sup> NH <sub>4</sub> <sup>+</sup>	369 375 —	— — 0.5	58 60 10	Wolcott and Wolcott, 1991
<i>Ocypode quadrata</i>	Na <sup>+</sup> Cl <sup>-</sup>	402 341	— —	60 52	Wolcott and Wolcott, 1985

isms in the laboratory to maintain a constant ionic milieu in the face of salinity changes fall short of values determined in the field or under more natural conditions (Flemister, 1958; Rabalais and Cameron, 1985; D'Orazio and Holliday, 1985; Zanders and Martelo, 1984; Gross, 1964; Morrill, 1988; Spicer *et al.*, 1987; Blasco and Forward, 1988). On the other hand, understanding the integrated response is not possible until the contributions of cellular and subcellular processes are appreciated. Integrated responses of many biochemical, neural, endocrinological and behavioural systems contribute to enantiostatic control of internal functions. Both the detailed, isolated mechanisms and the integrated systems must be studied before a clear picture of iono- and osmoregulation is achieved.

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#### LITERATURE CITED

- AL-ADHUB, A.H.Y. AND NAYLOR, E. 1975. Emergence rhythm and tidal migrations in the brown shrimp *Crangon crangon* (L.). *Journal of*

- the Marine Biological Association, United Kingdom 55: 801-810.
- BALLANTYNE, J.S., MOYES, C.D. AND MOON, T.W. 1987. Compatible and counteracting solutes and the evolution of ion and osmoregulation in fishes. *Canadian Journal of Zoology* 65: 1883-1888.
- BAYLISS, D. AND HARRIS, R.R. 1988. Chloride ion regulation in the freshwater amphipod *Corophium curvispinum* and acclimatory effects of external Cl<sup>-</sup>. *Journal of Comparative Physiology B* 158: 81-90.
- BIANCHINI, A. AND GILLES, R. 1990. Cyclic AMP as a modulator of NaCl transport in gills of the euryhaline Chinese crab *Eriocheir sinensis*. *Marine Biology* 104: 192-195.
- BIRCHARD, G.F., DROLET, L., AND MANTEL, L.H. 1982. The effect of reduced salinity on osmoregulation and oxygen consumption in the lady crab *Ovalipes ocellatus* (Herbst). *Comparative Biochemistry and Physiology* 71A: 321-324.
- BLASCO, E. AND FORWARD, R.B., JR. 1988. Osmoregulation of the xanthid crab, *Panopeus herbstii*. *Comparative Biochemistry and Physiology* 90A(1): 135-139.
- BOOTH, C.E. 1986. Apparent net H<sup>+</sup> flux and acid-base balance in the spider crab, *Libinia emarginata*. *American Zoologist* 26: 45A.
- BURNETT, L.E. 1988. Physiological responses to air exposure: Acid-base balance and the role of branchial water stores. *American Zoologist* 28: 125-135.
- BURNETT, L.E. AND CARROLL, A.K. 1989. Chloride ion and CO<sub>2</sub> fluxes across the isolated perfused gills of the blue crab. *American Zoologist* 29(4): 153A.
- BURNETT, L.E. AND McMAHON, B.R. 1987. Gas exchange, haemolymph acid-base status, and the role of branchial water stores during air exposure in three littoral crab species. *Physiological Zoology* 60(1): 27-36.
- BURNETT, L.E., WOODSON, P.J., RIETOW, M.G. AND VILICICH, V.C. 1981. Crab gill intra-epithelial carbonic anhydrase plays a major role in haemolymph CO<sub>2</sub> and chloride ion regulation. *Journal of Experimental Biology* 92: 243-254.
- CAMERON, J.N. 1978. Effects of hypercapnia on blood acid-base status, NaCl fluxes, and trans-gill potential in freshwater blue crabs, *Callinectes sapidus*. *Journal of Comparative Physiology* 123: 137-141.
1981. Brief introduction to the land crabs of the Palau Islands: stages in the transition to air breathing. *Journal of Experimental Zoology* 218: 1-5.
- CHAMBERLAIN, M.E. AND STRANGE, K. 1989. Anisotonic cell volume regulation: a comparative view. *American Journal of Physiology* 257: C159-C173.
- D'ORAZIO, S.E. AND HOLLIDAY, C.W. 1985. Gill Na, K-ATPase and osmoregulation in the sand fiddler crab, *Uca pugnator*. *Physiological Zoology* 58(4): 364-373.
- DEFUR, P.L., NUSBAUMER, D. AND LEWIS, R.J. 1988. Physiological aspects of molting in blue crabs from the tidal fresh-water Potomac River, Virginia. *Journal of Crustacean Biology* 8: 12-19.
- DENNE, L.B. 1968. Some aspects of osmotic and ionic regulation in the prawns *Macrobrachium australiense* and *M. equidens* (Dana). *Comparative Biochemistry and Physiology* 26: 17-30.
- EWING, R.D., PETERSON, G.L. AND CONTE, E.P. 1974. Larval salt gland of *Artemia salina* nauplii. Localisation and characterisation of the sodium + potassium activated ATPase. *Journal of Comparative Physiology* 88: 217-234.
- FLEMISTER, L.J. 1958. Salt and water anatomy, constancy and regulation in related crabs from marine and terrestrial habitats. *Biological Bulletin* 115: 180-200.
- FRANCO, P.L. AND AHEARN, G.A. 1989. Electrogenic 2Na<sup>+</sup>/1H<sup>+</sup> antiport in lobster antennal glands. *American Zoologist* 29(4): 152A.
- GILLES, R. 1987. Volume regulation in cells of euryhaline invertebrates. 205-247. In A. Kleinzeller (ed.) 'Cell volume control: Fundamental and comparative aspects in animal cells'. (Academic Press: New York).
- GILLES, R. AND GILLES-BAILLIEN, M. (eds). 1985. Transport processes, ionic- and osmoregulation. (Springer-Verlag: New York). 483p.
- GILLES, R. AND PEQUEUX, A. 1983. Interactions of chemical and osmotic regulation with the environment. 109-77. In F.J. Vernberg and W.B. Vernberg (eds) 'The biology of Crustacea, Vol. 8, Environmental adaptations'. (Academic Press: New York).
- GOOLISH, E.M. AND BURTON, R.S. 1989. Energetics of osmoregulation in an intertidal copepod: effects of anoxia and lipid reserves on the pattern of free amino acid accumulation. *Functional Ecology* 3: 81-89.
- GRAHAM, J.B. 1990. Ecological, evolutionary, and physical factors influencing aquatic animal respiration. *American Zoologist* 30: 137-146.
- GRASZYNSKI, K. AND BIGALKE, T. 1987. Osmoregulation and ion transport in the extremely euryhaline fiddler crabs *Uca pugnator*



- and *Uca tangeri* (Ocypodidae). Zoologische Beiträge 30: 339–358.
- GREENAWAY, P. 1988. Ion and water balance. 211–248. In W.W. Burggren and B.R. McMahon (eds) 'Biology of the land crabs'. (Cambridge University Press: Cambridge).
- GREENAWAY, P. AND MORRIS, S. 1989. Adaptations to a terrestrial existence by the robber crab, *Birgus latro* L. III. Nitrogenous excretion. Journal of Experimental Biology 143: 333–346.
- GROSS, W.J. 1964. Trends in water and salt regulation among aquatic and amphibious crabs. Biological Bulletin 127: 447–466.
- HARRIS, R.R. AND ANDREWS, M.B. 1985. Total NPS pool and ammonia net efflux rate changes in *Carcinus maenas* during acclimation to low environmental salinity. Comparative Biochemistry and Physiology 82A: 301–308.
- HENRY, R.P. 1988. Subcellular distribution of carbonic anhydrase activity in the gills of the blue crab *Callinectes sapidus*. Journal of Experimental Biology 245: 1–8.
- HENRY, R.P. AND CAMERON, J.N. 1982. The distribution and partial characterization of carbonic anhydrase in selected aquatic and terrestrial decapod crustaceans. Journal of Experimental Zoology 221: 309–321.
- HOCHACHKA, P.W. AND SOMERO, G.N. 1984. 'Strategies of biochemical adaptation'. (Princeton University Press: Princeton, NJ) 537p.
- HOFFMAN, E.K. 1986. Anion transport systems in the plasma membrane of vertebrate cells. Biochimica et Biophysica Acta 864: 1–31.
- HOFFMANN, E.K. AND SIMONSEN, L.O. 1989. Membrane mechanisms in volume and pH regulation in vertebrate cells. Physiological Reviews 69(2): 315–382.
- HOLLIDAY, C.W. 1985. Na, K-ATPase activity and hypoosmoregulation in the brine shrimp, *Artemia salina*. American Zoologist 25: 138A.
1985. Salinity-induced changes in gill Na, K-ATPase activity in the mud fiddler crab, *Uca pugnax*. Journal of Experimental Zoology 233: 199–208.
1988. Branchial Na<sup>+</sup>/K<sup>+</sup>-ATPase and osmoregulation in the isopod, *Idotea wosnesenskii*. Journal of Experimental Biology 136: 259–272.
- HOLLIDAY, C.W., ROYE, D.B. AND ROEK, R.D., 1990. Salinity-induced changes in branchial Na,K-ATPase activity and transepithelial potential difference in the brine shrimp, *Artemia salina*. Journal of Experimental Biology 151: 279–296.
- KAMEMOTO, F.I., KATO, K.N., AND TUCKER, L.E. 1966. Neurosecretion and salt and water balance in the annelida and crustacea. American Zoologist 6: 213–219.
- KIRSCHNER, I.B. 1979. Control mechanisms in crustaceans and fishes. 157–222. In R. Gille (ed.) Mechanisms of osmoregulation in animals. (Wiley-Interscience: New York). 667p.
- LOCKWOOD, A.P.M. 1961. The urine of *Gammarus duebeni* and *G. pulex*. Journal of Experimental Biology 38: 647–658.
- LUCU, C., DEVESCOVI, M. AND SIEBERS, D. 1989. Do amiloride and ouabain affect ammonia fluxes in perfused *Carcinus* gill epithelia? Journal of Experimental Zoology 249: 1–5.
- LUCU, C. AND SIEBERS, D. 1987. Linkage of Cl<sup>-</sup> fluxes with ouabain-sensitive Na/K exchange through *Carcinus* gill epithelia. Comparative Biochemistry and Physiology 87A: 807–811.
- MANGUM, C.P. 1986. Osmoregulation in marine and estuarine animals: its influence on respiratory gas exchange and transport. Bollettino di Zoologia, 53: 1–7.
- MANGUM, C.P. AND RAINER, J.S. 1988. The relationship between subunit composition and O<sub>2</sub> binding of blue crab haemocyanin. Biological Bulletin 174: 77–82.
- MANGUM, C.P., SILVERTHORN, S.U., HARRIS, J.L., TOWLE, D.W. AND KROLL, A.R. 1976. The relationship between blood pH, ammonia excretion, and adaptation to low salinity in the blue crab, *Callinectes sapidus*. Journal of Experimental Zoology 195: 129–136.
- MANTEL, L.H. AND FARMER, L.L. 1983. Osmotic and ionic regulation. 53–161. In L.L. Mantel (ed.) 'The biology of Crustacea, Vol. 5, Internal anatomy and physiological regulation'. (Academic Press: New York). 471p.
- MANTEL, L.H. AND OLSON, J.R. 1976. Studies on the Na<sup>+</sup>, K<sup>+</sup>-activated ATPase of crab gills. American Zoologist 16: 223A.
- MASON, R.P., MANGUM, C.P. AND GODETTE, G. 1983. The influence of inorganic ions and acclimation salinity on haemocyanin-oxygen binding in the blue crab *Callinectes sapidus*. Biological Bulletin 164: 104–123.
- MORRIS, C.E. AND SIGURDSON, W.J., 1989. Stretch-inactivated ion channels coexist with stretch-activated ion channels. Science 243: 807–809.
- MORRITT, D. 1988. Osmoregulation in littoral and terrestrial talitroidean amphipods (Crustacea) from Britain. Journal of Experimental Marine Biology and Ecology 123: 77–94.
- NEUFELD, G.J., HOLLIDAY, C.W. AND PRITCHARD, J.B. 1980. Salinity adaptation of gill Na,

- K-ATPase in the blue crab, *Callinectes sapidus*. *Journal of Experimental Zoology* 211: 215-224.
- PEQUEUX, A. AND GILLES, R. 1977. Osmoregulation of the chinese crab *Eriocheir sinensis* as related to the activity of the (Na<sup>+</sup>-K<sup>+</sup>)ATPase. *Archives Internationales de Physiologie et de Biochimie* 85: 41-42.
1988. The transepithelial potential difference of isolated perfused gills of the chinese crab *Eriocheir sinensis* acclimated to freshwater. *Comparative Biochemistry and Physiology* 89A: 163-172.
- PIERCE, S.K. 1982. Invertebrate cell volume control mechanisms: a coordinated use of intracellular amino acids and inorganic ions as osmotic solute. *Biological Bulletin* 163: 405-419.
- PINDER, A.W. AND SMITS, A.W. 1986. The burrow habitat of the land crab, *Cardisoma guanhumi*. *American Zoologist* 26(4): 49A.
- POTTS, W.T.W. AND DURNING, C.T. 1980. Physiological evolution in the branchiopods. *Comparative Biochemistry and Physiology* 67B: 475-484.
- PRESSLEY, T.A., GRAVES, J.S. AND KRALL, R. 1981. Amiloride-sensitive ammonium and sodium ion transport in the blue crab. *American Journal of Physiology* 241: 370-378.
- PRITCHARD, A.W. AND KERLEY, D.E. 1970. Kidney function in the north American crayfish *Pacifastacus leniusculus* (Dana) stepwise acclimated to dilutions of sea water. *Comparative Biochemistry and Physiology* 35: 427-437.
- RABALAIS, N.N. AND CAMERON, J.N. 1985. Physiological and morphological adaptations of adult *Uca subcylindrica* to semi-arid environments. *Biological Bulletin* 168: 135-146.
- RANDALL, D.J. AND WOOD, C.M. 1981. Carbon dioxide excretion in the land crab (*Cardisoma carnifex*). *Journal of Experimental Zoology* 218: 37-44.
- REGNAULT, M. 1987. Nitrogen excretion in marine and freshwater crustacea. *Biological Reviews* 62: 1-24.
- RIEGEL, J.A. 1968. Analysis of the distribution of sodium, potassium and osmotic pressure in the urine of crayfish. *Journal of Experimental Biology* 48: 587-596.
- SACKIN, H. 1987. Stretch-activated potassium channels in renal proximal tubule. *American Journal of Physiology* 253: F1253-F1262.
- SIEBERS, D., LUCU, C., WINKLER, A., DALLA VENEZIA, L. AND WILLE, H. 1986. Active uptake of sodium in the gills of the hyperregulating shore crab *Carcinus maenas*. *Helgolander Meeresunters* 40: 151-160.
- SIEBERS, D., WILLE, H., LUCU, C. AND DALLA VENEZIA, L. 1989. Conductive sodium entry in gill cells of the shore crab, *Carcinus maenas*. *Marine Biology* 101: 61-68.
- SPAARGAREN, D.H. 1982. The ammonium excretion of the shore crab, *Carcinus maenas*, in relation to environmental osmotic conditions. *Netherlands Journal of Sea Research* 15(2): 273-283.
- SPAARGAREN, D.H., RICHARD, P. AND CECALDI, H.J. 1982. Excretion of nitrogenous products by *Penaeus japonicus* Bate in relation to environmental osmotic conditions. *Comparative Biochemistry and Physiology* 72A: 673-678.
- SPICER, J.I., MOORE, P.G. AND TAYLOR, A.C. 1987. The physiological ecology of land invasion by the Talitridae (Crustacea: Amphipoda). *Proceedings of the Royal Society of London B* 232: 95-124.
- STEWART, P.A. 1978. Independent and dependent variables of acid-base control. *Respiration Physiology* 33: 9-26.
- TAYLOR, A.C., SPICER, J.I. AND PRESTON, T. 1987. The relationship between osmoregulation and nitrogen metabolism in the intertidal prawn *Palaemon elegans* (Rathke). *Comparative Biochemistry and Physiology* 88A: 291-298.
- TAYLOR, P.M. AND HARRIS, R.R. 1986. Osmoregulation in *Corophium curvispinum* (Crustacea: Amphipoda), a recent coloniser of freshwater. I. Sodium ion regulation. *Journal of Comparative Physiology* 156B: 323-329.
- THOMPSON, W.E., MOLINARO, P.J., GRECO, T.M., TEDESCHI, J.B. AND HOLLELIDAY, C.W. 1989. Regulation of haemolymph volume by uptake of sand capillary water in desiccated fiddler crabs, *Uca pugilator* and *Uca pugnax*. *Comparative Biochemistry and Physiology* 94A: 531-538.
- THUET, P., CHARMANTIER-DAURES, M. AND CHARMANTIER, G. 1988. Relation entre osmoregulation et activites d'ATPase Na<sup>+</sup>-K<sup>+</sup> et d'anhydrase carbonique chez larves et postlarves de *Homarus gammarus* (L.) (Crustacea: Decapoda). *Journal of Experimental Marine Biology and Ecology* 115: 249-261.
- TOWLE, D.W. 1984. Membrane-bound ATPases in arthropod ion-transporting tissues. *American Zoologist* 24: 177-185.
- TOWLE, D.W. AND BAKSINSKI, A. 1989. Electrogenicity of the crustacean Na<sup>+</sup>/H<sup>+</sup> exchanger demonstrated with a potential-sensitive dye. *American Zoologist* 29(4): 152A.
- TOWLE, D.W. AND HOLLELAND, T. 1987. Ammonium ion substitutes for K<sup>+</sup> in ATP-dependent Na<sup>+</sup> transport by basolateral membrane vesicles.

- American Journal of Physiology **252**: R479–R489.
- TOWLE, D.W. AND KAYS, W.T. 1986. Basolateral localization of  $\text{Na}^+ + \text{K}^+$ -ATPase in gill epithelium of two osmoregulating crabs, *Callinectes sapidus* and *Carcinus maenas*. Journal of Experimental Zoology **239**: 311–318.
- TOWLE, D.W., PALMER, G.E. AND HARRIS, J.L. III 1976. Role of gill  $\text{Na}^+ + \text{K}^+$ -dependent ATPase in acclimation of blue crabs (*Callinectes sapidus*) to low salinity. Journal of Experimental Zoology **196**: 315–322.
- TRUCHOT, J.-P. 1973. Fixation et transport de l'oxygene par le sang de *Carcinus maenas*: variations en rapport avec diverses conditions de temperature et de salinite. Netherlands Journal of Sea Research **7**: 482–495.
1981. The effect of water salinity and acid-base state on the blood acid-base balance in the euryhaline crab, *Carcinus maenas* (L.). Comparative Biochemistry and Physiology **68A**: 555–561.
- WANSON, S.A., PEQUEUX, A.J.R. AND ROER, R.D. 1984.  $\text{Na}^+$  regulation and  $(\text{Na}^+ + \text{K}^+)$ -ATPase activity in the euryhaline fiddler crab *Uca minax* (Le Conte). Comparative Biochemistry and Physiology **79A**: 673–678.
- WEILAND, A.L. AND MANGUM, C.P. 1975. The influence of environmental salinity on haemocyanin function in the blue crab *Callinectes sapidus*. Journal of Experimental Zoology **193**: 265–274.
- WHEATLY, M.G. AND MCMAHON, B.R. 1982. Responses to hypersaline exposure in the euryhaline crayfish *Pacifastacus leniusculus* I. The interaction between ionic and acid-base regulation. Journal of Experimental Biology **99**: 425–445.
- WHEATLY, M.G. AND TOOP, T. 1989. Physiological responses of the crayfish *Pacifastacus leniusculus* to environmental hyperoxia II. Role of the antennal gland in acid-base and ion regulation. Journal of Experimental Biology **143**: 53–70.
- WOLCOTT, D.L. IN PRESS. Nitrogen excretion is enhanced during urine recycling in two species of terrestrial crab. Journal of Experimental Zoology.
- WOLCOTT, T.G. 1973. Physiological ecology and intertidal zonation in limpets (*Acmaea*): a critical look at 'limiting factors'. Biological Bulletin **145**: 389–422.
- WOLCOTT, T.G. AND WOLCOTT, D.L. 1985. Extrarenal modification of urine for ion conservation in ghost crabs, *Ocypode quadrata* (Fabricius). Journal of Experimental Marine Biology and Ecology **91**: 93–107.
1988. Availability of salts is not a limiting factor for the land crab, *Gecarcinus lateralis* (Fremenville). Journal of Experimental Marine Biology and Ecology **120**: 199–219.
1991. Ion conservation by reprocessing of urine in the land crab *Gecarcinus lateralis* (Fremenville). Physiological Zoology **64**: 344–361.
- YANCEY, P.H., CLARK, M.E., HAND, S.C., BOWLUS, R.D. AND SOMERO, G.N. 1982. Living with water stress: evolution of osmolyte systems. Science **217**: 1214–1222.
- ZANDERS, I.P. AND MARTELO, M.-J. 1984. Influence of temperature on ionic regulation in the mangrove crab *Goniopsis cruentata*. Comparative Biochemistry and Physiology **78A**(2): 249–254.
- ZANDERS, I.P. 1978. Ionic regulation in the mangrove crab *Goniopsis cruentata*. Comparative Biochemistry and Physiology **60**: 293–302.